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TITLE: Prospective Evaluation of Intraprostatic Inflammation and Focal Atrophy as a Predictor of Risk of High-Grade Prostate Cancer and Recurrence after Prostatectomy

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14. ABSTRACT

We are evaluating, in two nested case-control studies, intraprostatic inflammation and focal atrophy, a prostate lesion that is often inflamed, as tissue markers for risk of future diagnosis of total and high-grade prostate cancer, and for prognosis at the time of surgery for clinically localized prostate cancer. For prostate cancer incidence, in Year 3, we completed the statistical analysis of the pathology review of inflammation and focal atrophy the H&E stained biopsy core images for the linked PCPT-SELECT data (incident prostate cancer). We found that the odds of low-grade prostate cancer tended to increase with the number of biopsy cores with inflammation. For prostate cancer recurrence, we used an optimized higher-throughput method for image analysis to evaluate in the Brady nested case-control recurrence set the association between mast cell density and recurrence risk. We found that greater mast cell density in tumor was inversely associated with recurrence, while greater density in normal tissue was positively associated with recurrence. We received a 1- year Extension without Funds (end date 6/30/2016) to be able to complete all tasks for our promising work.

15. SUBJECT TERMS

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1. INTRODUCTION:

With respect to healthy men, at this time, we do not know how to prevent the development of prostate cancer that has the potential to be aggressive, nor do we have a tool to identify men who would most benefit from preventive interventions for aggressive disease. With respect to men with early prostate cancer, at this point, we still cannot predict with certainty which men are more likely to suffer and die of their prostate cancer after prostatectomy. In this population-based research project, we are directly addressing these major problems. We are evaluating, in two nested case-control studies, intraprostatic inflammation and focal atrophy, a prostate lesion that is often inflamed, as tissue markers for risk of future diagnosis of total and high-grade prostate cancer, and for prognosis at the time of surgery for clinically localized prostate cancer. Our overall hypotheses are: 1) Chronic intraprostatic inflammation is a cause of prostate cancer that is more likely to be aggressive and recur. 2) Focal atrophy, a prostate lesion that is often inflamed, is a risk and prognostic indicator.

2. KEYWORDS:

Prostate cancer, risk, incidence, recurrence, inflammation, focal atrophy, mast cells, immune cells, tissue microarray, biopsy, image analysis, odds ratio.

3. OVERALL PROJECT SUMMARY:

Prostate cancer incidence:

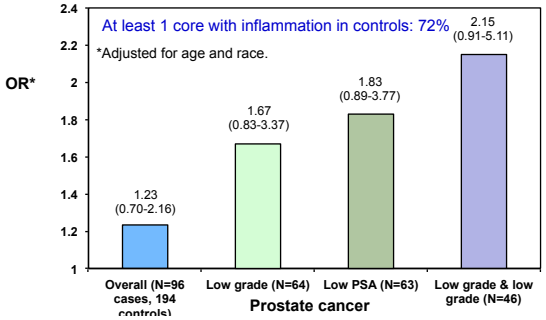
For Aims 1 and 2, we completed **Tasks 1, 2, 3a-c, 4a-b, f** in Years 1 and 2. In Year 3, we on-boarded the new biostatistician mentioned in the last progress report (arrived in 8/2014) and trained him for these analyses, we cleaned the merged pathology-PCPT-SELECT data from the review of the H&E stained slide images **(Task 7a)**, and performed the statistical analysis of the merged data to address the association of inflammation (Aim 1) and focal atrophy (Aim 2) with prostate cancer risk **(Task 7b)**, and began to draft the manuscript for inflammation and risk **(Task 7d)**. We have presented these findings in talks and small prostate-cancer meetings for initial feedback **(Task 7c)**. We are currently IHC staining the PCPT-SELECT biopsy cores **(Task 3e,f).** Over the next and final year, we will complete the staining**,** will image these slides and upload into TMAJ **(Task 4c,d)**, and perform data cleaning and statistical analysis **(Task 7a,b for IHC)**.

Prostate cancer recurrence: For Aims 3 and 4, we completed **Tasks 1, Task 5a-c, Task5 d-e** (for mast cells), we optimized and implemented a more efficient method of image analysis for IHC-stained TMA sections and documented its accuracy relative to manual counting for mast cells and optimized double stains for CK8 and CD4, CD8, CD20, CD68, and FoxP3 **(Relevant to Task 3f** and **Task 5e)** in Years 1 and 2. In Year 3, we completed image analysis for mast cell numbers and total epithelial area with the PIP software (**Task6c** for mast cells), merged the data with the Brady recurrence database and cleaned the merged data (**Task 8a** for mast cells) and performed the statistical analysis (**Task 8b** for mast cells), presented some of the results at a national meeting (Hempel HA et al. AACR 2015, Philadelphia, PA); **Task 8c** for mast cells), and began drafting a manuscript (**Task 8d** for mast cells). We prepared this progress report **(Task 6f)**. Over the next and final year, we will perform the review of the H&E stained images for the recurrence set for the prevalence and extent of inflammation and focal atrophy **(Task 6a-b)**, complete staining and imaging all of the TMA sections for the remaining markers **(Task 5c,d),** use PIP and other methods to count the number of cells staining positive **(Task 6c,d)**, and perform data cleaning and statistical analysis **(Task 8a,b)**.

KEY RESEARCH ACCOMPLISHMENTS:

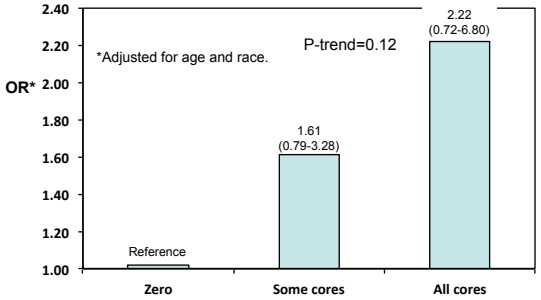
Prostate cancer incidence: The final data included 96 prostate cancer cases and 194 controls frequency matched on age and race. Reported here are the chief results from Aim 1. Of the cases, Gleason sum was known for 76%; only 7 were of higher Gleason sum. Cases and controls did not significantly differ in their characteristics except for family history of prostate cancer and daily intake of energy and macronutrients. Demographic, anthropometric, and dietary characteristics of the controls were generally not associated with extent of inflammation. We observed that 72% of

Figure 1. Association between any biopsy core with inflammation and prostate cancer, PCPT-SELECT linked



controls had at least one biopsy core with inflammation. This percentage was modestly lower than what we previously reported in a different group of controls sampled from the PCPT (78%; PMID: 24748218). Overall, having at least 1 biopsy core with inflammation was not associated with risk of prostate cancer. However, it appeared that having at least 1 biopsy core with inflammation was positively associated with low-grade prostate cancer, especially when restricting to men who also had low PSA (Figure 1). The association for prostate cancer overall was null. Risk of low-grade prostate cancer tended to increase with the number of biopsy cores with inflammation (Figure 2). While not statistically significant, this first prospective study of men without indication for biopsy provides some evidence in support of the hypothesis that inflammation influences the development of prostate cancer. Interestingly, the magnitude of the association for low-grade disease is similar to what we observed previously in the PCPT using a non-temporally clear study design (at least 1 core with inflammation: OR=1.57, 95% CI 0.83-3.00; PMID: 24748218). In that prior study, we noted a stronger association for high-grade disease (OR=2.24, 95% CI 1.06–4.71; p-trend across none, some, all cores with inflammation = 0.01). Our PCPT-SELECT study does not rule out an association with high-grade disease; we could not address the association for higher-grade disease because of small numbers.

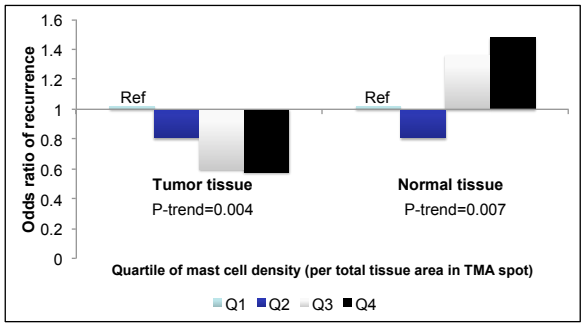
Figure 2. Association between extent of biopsy cores with inflammation and low-grade prostate cancer, PCPT-SELECT linked



Reported here is the key result from Aim 2. Focal atrophy, the majority of which was simple atrophy, was not clearly associated with an increased (as hypothesized) risk of prostate cancer (at least 1 core positive: OR=0.84, 95% CI 0.40-1.80) or low-grade disease (OR=0.88, 95% CI 0.37-2.13). Our temporally clear study does not support an association between focal atrophy and prostate cancer risk, results that are consistent with our less temporally clear analysis in PCPT (prostate cancer: OR=0.90, 95% CI 0.44-1.83, low-grade disease: OR=0.85, 95% CI 0.36-2.05; in process).

Prostate cancer recurrence: Here we report on the exciting finding for mast cells, a component of the innate immune system. Final data included 462 men who recurred (cases) and 462 men matched to the cases who did not recur (controls) from the Brady nested case-control study of recurrence. We quantified mast cells and epithelial area in the tissue microarray (TMA) spots by PIP digital image analysis (the method integrates whole slide imaging, virtual microscopy, and ImageJ based analysis algorithms). In a prior reporting year, we documented that the counts for IHC-positive cells are comparable to manual assessment for mast cells. We used conditional logistic regression to estimate ORs and 95% CIs of recurrence for minimum mast cell density (number of mast cells per total TMA spot area) among each man's cancer or benign TMA spots. Mast cell density was significantly higher in cancer areas than in benign areas (p=0.0016). In controls, higher mast cell density in tumor (p-trend<0.02), but not benign tissue (p-trend=0.7), was inversely associated with higher-grade disease (Gleason score 4+3 or higher). After taking into account Gleason score as well as age, race, and pathologic stage, higher mast cell density per tissue area in the tumor was inversely associated with recurrence (comparing highest to lowest quartiles: OR=0.58, 95% CI 0.40-0.86, p-trend=0.004; Figure 3). In contrast, higher mast cell density per tissue area in normal appearing tissue from these men with prostate cancer was positively associated with risk of recurrence (OR=1.48, 95% CI 1.02-2.14, p-trend=0.0007; Figure 3).

Figure 3. Association between mast cell density in tumor or normal tissue and risk of prostate cancer recurrence, Brady nested case-control study



As we indicated in our prior progress report, through this work, we developed a resource for prostate cancer researchers: a new cohort derived from the linkage of the PCPT and SELECT trials. This cohort consists

of men who were negative for prostate cancer on PCPT end-of-study biopsy and who then enrolled in SELECT. Linking these 2 cohorts is the ONLY epidemiologically sound approach for prospectively testing the association of tissue markers in men without an indication for biopsy or surgery with prostate cancer incidence – at this time and in the foreseeable future. Access to this linked resource is via SWOG (http://swog.org/Visitors/Biorepository/).

5. CONCLUSION:

Aims 1 and 2: Taken together, our findings from the temporally clear analysis in PCPT-SELECT (inflammation was assessed in tissue removed by biopsy in months to years before the diagnosis of prostate cancer) and the less temporally clear analysis in PCPT (inflammation was assessed in the tissue reviewed to make or exclude the diagnosis of prostate caner) that we previously conducted (PMID: 24748218) support an etiologic role of intraprostatic inflammation in the development of prostate cancer. Our PCPT-SELECT study does not rule out an association with high-grade disease (the strongest association we observed in the PCPT); we could not address the association for higher-grade disease because of small numbers. Our studies support the conduct of additional studies to investigate the specific immune cell milieu of the prostate that may be associated with the development of an aggressive prostate cancer phenotype. These studies are underway in PCPT-SELECT as part of this DOD award. Taken together, our findings from our present and prior studies do not support that focal atrophy is associated with an increased risk of prostate cancer, as we had hypothesized. Aim 3: Our results suggest that mast cells within the tumor may protect against recurrence, or could serve as a marker for the likelihood of a tumor to recurrence, whereas mast cells elsewhere in the tissue may influence the progression of this cancer.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Publications: None to date. Manuscripts are currently being drafted for Aim 1 inflammation and prostate cancer risk and Aim 3 mast cells and prostate cancer recurrence.

Presentations and abstracts:

Talks in which data from Aim 1 were presented:
"Inflammation and prostate cancer". Joint Meeting of the Johns Hopkins Prostate Cancer SPORE and the Thomas Jefferson/University of Pennsylvania Prostate Cancer SPORE. November 7, 2014, Philadelphia, PA.

"Updates on the epidemiology of prostate cancer and BPH/LUTS". Prostate Research Day, Johns Hopkins University (attendees include Prostate Cancer Advisory Board members and SPORE External Advisors [e.g., including Peter Gann, Howard Soule, Eric Klein, Howard Scher], as well as JH researchers), February 21, 2015.

"Successes in working together to identify modifiable risk factors and tissue-based markers: prostate cancer risk and recurrence". Ohio State University James Cancer Center, December 10, 2014, and Department of Hygiene and Epidemiology, University of Ioannina, Ioannina, Greece, July 7, 2015.

Poster in which data from Aim 3 were presented:
Abstract 2342: Characterization of inflammatory markers and mast cells in association with prostate cancer. Heidi Hempel, Ibrahim Kulac, Nathan S. Cuka, Toby C. Cornish, Elizabeth A. Platz, Angelo M. DeMarzo, Karen S. Sfanos. American Association for Cancer Research Annual Meeting, April 20, 2015, Philadelphia, PA.

7. INVENTIONS, PATENTS AND LICENSES: None

8. REPORTABLE OUTCOMES: None to date.

9. OTHER ACHIEVEMENTS:

As we indicated in our prior progress report, through this work, we developed a resource for prostate cancer researchers: a new cohort derived from the linkage of the PCPT and SELECT trials. This cohort consists of men who were negative for prostate cancer on PCPT end-of-study biopsy and who then enrolled in SELECT. Linking these 2 cohorts is the ONLY epidemiologically sound approach for prospectively testing the association of tissue markers in men without an indication for biopsy or surgery with prostate cancer incidence – at this time and in the foreseeable future. Access to this linked resource is via SWOG (http://swog.org/Visitors/Biorepository/).

The mast cell research in Aim 3 is led by Heidi A. Hempel, a doctoral candidate at the Johns Hopkins School of Medicine; this work on mast cells and recurrence forms a component of dissertation. Her advisor is Karen Sfanos, Assistant Professor at the Johns Hopkins School of Medicine, a collaborator of Drs. Platz and De Marzo on the basic science of infectious agents and resultant inflammation in the etiology of prostate cancer.

REFERENCES: None

APPENDICES: None

SUPPORTING DATA: None